BIOCONTROL POTENTIAL OF *TRICHODERMA VIRIDE* ON SOYBEAN (*GLYCINE MAX* L.) IN MID HILL CONDITIONS OF HIMACHAL PRADESH

SYNOPSIS OF THESIS

Submitted By

Manjula

Registration No. AU21AGPhD0077

Under the Supervision of

Dr. Ajay Kumar Gautam

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF AGRICULTURE

FACULTY OF AGRICULTURE

ABHILASHI UNIVERSITY

CHAILCHOWK, CHACHYOT MANDI (H.P.)-175045

SEPTEMBER 2023

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DECLARATION

I hearby that the dissertation synopsis entitled "**Bio-control potential of *Trichoderma viride* on Soybean (*Glycine max* L.) in mid hill conditions of Himachal Pradesh.**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles, it has not been submitted to any other University or institution for the award of any degree or diploma.

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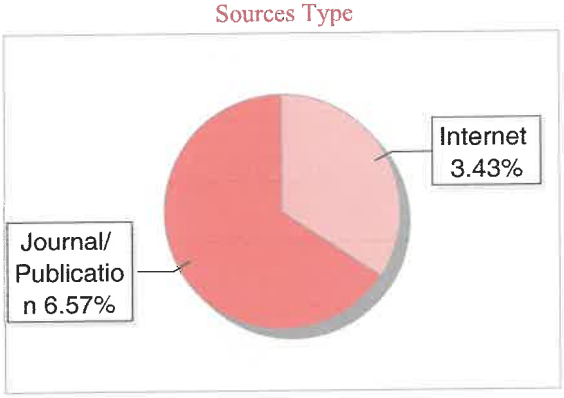

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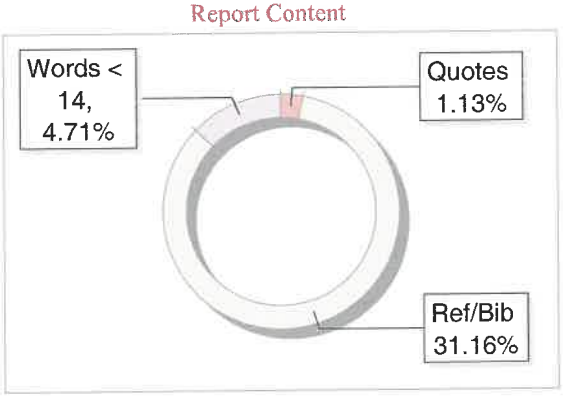

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ABHILASHI UNIVERSITY

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Synopsis of Thesis of Post Graduate Student

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Minor field	: 1. Genetics and Plant breeding 2. Soil Science
Title of the research problem	:"Biocontrol potential of *Trichoderma viride* on Soybean (*Glycine max* L.) in mid hill conditions of Himachal Pradesh".

1. Introduction

Soybean (*Glycine max* L.) is one of India's most important crop and a good source of vegetable protein and oil (Herridge *et al.* 2008; Prevost *et al.* 2010). Soybean is a leguminous oil seed crop grown all over the world as an excellent source of protein (40%) and fat (20%). Soybean is a subtropical plant that is native to Southeast Asia. In the 1700s and 1800s, this crop was introduced to Europe and the United States. Farmers in the Midwest of the United States produce roughly half of all soybeans. Around 45% of soybean production occurs in East Asia, with the remaining 55% occurring in America. Brazil, Argentina, Paraguay, China, and India are the top soybean producers, in addition to the United States. It is grown as a rainfed kharif crop in India on an area of 10.97 million ha, with a total production of 10.99 million tonnes and a productivity of 1002 kg/ha in 2016-17 (SOPA). Madhya Pradesh is India's leading state in soybean area and production, with 54.01 lakh ha under soybean production, an average productivity of 1020 kg/ha, and total production of 55.06 lakh tonnes in 2016-17 (SOPA).

Soybean is extremely sensitive to biotic and abiotic stresses, particularly plant pathogens, which can infect crop plants at various stages of development, from seed germination to physiological maturity. More than 100 pathogens have been reported to affect soybean, 35 of which are economically significant (Sinclair and Backman, 1989).

Diseases reduce yield by 10 to 30% on average in most production areas (Sinclair, 1994). South Dakota soybean fields are infested with a variety of foliar, stem, and root fungal diseases. Fungal and fugal-like diseases can cause significant losses under certain conditions. Among the fungi that can infect soybeans are Rhizoctonia root rot, Sclerotinia stem rot (white mold), Septoria leaf spot (brown spot), brown stem rot, charcoal rot, damping-off, powdery mildew, frogeye leaf spot, Fusarium root rot, Phytophthora root and stem rot, Cercospora blight, leaf spot, and purple seed stain. As a major cash crop in India, it is critical to control diseases in a timely manner in order to improve overall growth and production of the soybean crop. The harmful impact of chemical pesticide use is no longer hidden from anyone, so the use of biopesticides is increasing day by day. Worldwide, research into the development of new biopesticides is ongoing. Among biopesticides, the use of biocontrol agents is an environmentally friendly method of controlling various plant diseases. *Trichoderma* is a well-known biocontrol agent used to combat a variety of disease pathogens.

Trichoderma is a saprophytic fungus found on the roots of many plants. According to Siddiquee *et al.* (2012) and Meena *et al.* (2017), it is an antagonistic fungus that makes a variety of volatile and non-volatile organic compounds as well as diffusible antibiotics like trichodermin, gliotoxin, and viridin (Sharma *et al.* 2016) to control plant pathogenic fungi. During antagonistic activity, this fungi primarily compete with pathogenic fungi for nutrients and available space. Similar to bacteria, these fungi, like *Trichoderma*, colonize plant roots and give them the ability to protect against biotic stresses like pathogenic infections and promote plant growth. In addition to biotic stress defense, *Trichoderma* also reduces abiotic stress like salinity and drought stress. *Trichoderma* strains affect the synthesis of bioactive metabolites. *Trichoderma* species also improve the availability of nutrients for plants, aiding them in their ability to withstand a variety of biotic and abiotic stresses. *Trichoderma* species have been applied to leguminous plants as biocontrol agents and plant growth stimulants. In chickpea (Cicer arietinum) coinoculated with *T. harzianum* and *Aspergillus niger*, significant increases in shoot and root length and weight were seen. Keeping in view the above facts, the present experiment entitled "Biocontrol potential of *Trichoderma viride* for major diseases of Soybean (*Glycine max* L.) in mid hill conditions of Himachal Pradesh" will be undertaken at a Research farm of Abhilashi University Mandi (H.P.) with following objectives.

2. Objectives

a) To study the effects of *Trichoderma viride* on major disease of Soybean (*Glycine max* L.) in mid hill conditions of Himachal Pradesh,

b) to evaluate the effects of *Trichoderma viride* on growth parameters of Soybean (*Glycine max* L.) in mid hill conditions of Himachal Pradesh.

3. A brief resume of work done

The soybean (*Glycine max* L.) crop is extremely susceptible to climate change and is adversely affected by it. As a result, the productivity is impacted by the climate, the prevalence of diseases, and insects in different years. Given that soybeans make up half of the area grown for legumes worldwide, India quickly became a major producer of soybeans

for export. In terms of avreage and output, it has held the top spot among oil seed crops. However, this increased production is affected in different years due to the influence of unfavorable climatic conditions, the occurrence of diseases and insects, and other factors, resulting in a significant loss of soybean production. Among variable constraints, fungal diseases are the most significant impediment to obtaining a high yield of soybean during commercial cultivation, and chemical fungicides are commonly used to control these diseases. The use of biocontrol agents such as *Trichoderma* is now thought to be a potential biocontrol and growth promoter for many crop plants, including soybean (Verma *et al.* 2007, Bai *et al.* 2008, and Savazzini *et al.* 2009). It is a well-documented biocontrol agent that has been shown to provide systemic protection against many seed-borne foliar diseases. This chapter contains a detailed review of the biocontrol potential of *Trichoderma viride* on Soybean.

a) Work done in abroad

The use of *Trichoderma* as a biological control agent of phytopathogens was first reported in Brazil in 1950 against tobacco mosaic virus (Forster, 1950). Further research into its ability to control plant pathogenic fungi and oomycetes led to the development of commercial *Trichoderma* based products (Bettiol *et al.* 2012). Among them, bioproducts suggested for the management of the *Sclerotinia sclerotiorum* (Lib.) de Bary, the causative agent of stem rot disease, merit special attention (Meyer, 2015). One of the most important diseases affecting soybean crop production is sclerotinia stem rot (SSR). Water-soaked irregular spots are the first signs, which develop into brown lesions and eventually necrosis, wilting leaves, and plant death. On infected tissues, a distinctive mycelium that resembles white cotton can be seen. Sclerotia, which are the fungus's survival structures, form on hosts at a later stage of the disease's development. The most frequent source of the pathogen's spread is seed lots containing fungus mycelia or sclerotia. The disease can reduce soybean yields by up to 70% in favorable climate conditions (Meyer, 2015).

Some fungi, including *Trichoderma* and *Gliocladium* species, have shown promise in the biological control of the pathogens. Numerous studies have been conducted on the antagonistic activity of *Trichoderma* species against plant pathogens (Hjelijord *et al.* 2001 and Krause *et al.* 2001). There are several commercial formulations based on *Trichoderma harzianum* and *T. virens* that are available to control soil-borne and foliar diseases in a variety of horticultural crops (Harman *et al.* 1990, Lumsden *et al.* 1992, and Samuels *et al.* 1996), but there is little information on the effectiveness of *Trichoderma* isolates on charcoal rot of soybean in Iranian conditions.

b) Work done in India

Thakur and Norris (1928) reported the first isolation of *Trichoderma* species, which are now widely used in agricultural applications due to their well-known biological control mechanism. *Trichoderma* accounted for up to 3% of total fungal propagule in a variety of forest soils and 1.5% in pasture soils across a variety of crops (Domsch *et al. 1980). Trichoderma* species have been used as bio-control microbes for about 70 years to produce

various antimicrobial products that affect other microbes by parasitizing on other fungi (Weindling, 1934).

Wells *et al.* (1972) used this species inoculum along tomato seedling rows to protect the crop from *S. rolfsii*. It has also been shown to have biocontrol potential against *Macrophomina phaseolina* (Elad *et al.* 1982), *F. oxysporum f.sp. vasinfectum, F.culmorum,* and *F. oxysporum f.*sp. radius lycopersici, the causal agents of cotton seedling blight, melon, wheat, and tomato crown rot (Sivan and Chet, 1986 and Sivan *et al.* 1987). *T. harzianum* has also shown evidence of its ability to control the growth of several other pathogens, including *F. solani* (Calvet *et al.* 1990), *F. oxysporum f.sp. cicero, Pythium* aphanidermatum (Bhardwaj and Gupta, 1987), *Rhizoctonia solani* (Wilson et al. 1988, Cole and Zrinyka, 1988), and *S.rolfsii* (Deve and Dutta, 1988). *T. koningii* has demonstrated biocontrol potential against *U. segetum tritici* and *Drechslera sorokiniana, T. reesi* against *D. sorokiniana,* and *T. pseudo koningii* against karnal bunt among other *Trichoderma species.*

According to research by Prasad *et al.* (1978) and Kirvoshchakova and Mishchank (1990), *T. viride* is antagonistic to *Drechslera sorokiniana,* the causative agent of wheat root rot, seedling blights, and foliar blights.

According to Singh *et al.* (1991), the culture filtrate of *T. viride* significantly reduced the germination of teliospores and sporidia of *N. indica.*

When Mostafa (1993) used this biocontrol agent on barley seeds, it reduced *Helminthosporium* infection on coleoptiles by 87%.

Field tests against loose smut of wheat also demonstrated the biocontrol potential of this agent (Aggarwal *et al.* 1992).

It has been demonstrated that *T. harzianum* inhibits the growth of *S. rolfsii*, the accidental cause of root rot in a variety of crops.

c) Work done in Himachal Pradesh

In field experiments, Singh *et al.* (2000) found that *T. viride* and *T. harzianum* reduced the infection of *S. rolfsii* on tomato and peanut as well as R. *solani* on beans, cotton, potato, and other vegetables. When Arya and Kaushik (2001) tested the effectiveness of six species of *Trichoderma, G. virens*, and *Chaetomium globosum* against *F. oxysporum* and *R. solani*, they found that *G. virens* and *T. harzianum* showed the greatest inhibition.

According to Kumar and Sharma's (2011) study of the growth patterns of *Trichoderma* isolates, the conidial wall pattern and shape were smooth and globose to abovoid in *T. viride* and rough and sub-globose in *T. harzianum.*

According to Patil *et al*.(2012*)* using the antagonist reduced pathogen inhibition by a notable percentage when using the variable culture technique. Four different *Phythium* species that cause tomato plant root rot were discovered macro and microscopically characterized. Volatile metabolites, as opposed to diffusible metabolites, produce the most acceptable and significant results when used by *Trichoderma* spp. against test pathogens. These results can then be used as a bio-control tool for seed infection.

4. Technical program of work

a. Survey and assessment of disease samples:

In the soybean-growing regions of the mid-hill regions of the district Mandi in Himachal Pradesh, periodic surveys will be conducted. The survey will be conducted on a regular basis during the growing seasons of crops. Diseased soybean plants will be collected during the survey in labelled paper bags and brought to the lab for immediate isolation and pathogen identification.

b. Assessment of disease incidence (DI) and disease severity (DS):

The subsequent observation will be noted for the evaluation of disease incidence and disease severity. Surveys will be conducted periodically in soybean-growing areas in various localities to assess the disease incidence (percentage of plants infected) and disease severity (DS). The following formulas will be used to determine the percent disease incidence and severity:

$$\text{Disease incidence (PDI)} = \frac{\text{Total number of infected plant}}{\text{Total number of plant assessed}} \times 100$$

$$\text{Disease severity (PDS)} = \frac{\text{Some of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

4.1. Field trial:

To evaluate the effects of *Trichoderma viride* potential for bio-control on soybean (*Glycine max* L.) in mid-hill conditions in Himachal Pradesh, a field trial will be carried out at the research farm of the School of Agriculture, Abhilashi University, Mandi (H.P.). The following are the specific treatments that will be used in the current study:

A. Seed Treatments

Treatment details:

T_1 : Seed treatment by *Trichoderma viride* + FYM
T_2 : Seed treatment by *Trichoderma viride* + Vermicompost
T_3 : Seed treatment by *Trichoderma viride* + Neem cake
T_4 : Seed treatment by *Trichoderma viride* + Mustard cake
T_5 : Seed treatment by *Trichoderma viride* + *Pseudomonas fluorescens*
T_6 : Seed treatment by *Trichoderma viride*
T_7 : Control (no manure or no fertilizer)

B. Experimental Details:

Design	: Randomized block design
Variety	: Braag
Treatments	: 07
Replications	: 03
Experiment year	: 2023
Time of Sowing	: June
Seed rate	: 75kg/ha
Spacing	: 30 ×10 cm
Total plot	: 21
Plot size	: 4 m^2

4.2. Experimental methods:

a) Biological seed treatment:

Trichoderma viride talc-based formulation will be used for seed treatment. Weighing and moistening treated seeds with tap water will be done. The seeds will be gently mixed with bio-agents after the extra water has been drained from them.

b) Sowing of seeds:

With a hand harrow, the seeds will be sown at a depth of about 3 to 4 cm. Each plot will have seven rows that are 30 cm apart, with a minimum plant-to-plant distance of 10 cm. Each furrow will be manually seeded, then immediately covered with soil.

c) Weeding:

The crop will primarily be grown using rainwater. The experimental field will undergo two manual hand weedings to keep it clear of weeds.

4.3. Observations to be recorded:

I. Crop Studies (Soybean) Growth parameters

a) Rate of germination and sruvival

Out of all the seeds sown, the total number of seeds that germinated and survived will be calculated and expressed as a percentage of germination for each treatment and replication.

b) Shoot length

The plant's height will be measured from the ground to the tip of the apical bud. Plant height will be measured beginning with germination and continuing at 30, 60, 90, and so on until harvesting. Ten plants will be chosen from each plot for this purpose, and each plant's height will be measured from the soil's surface to the tip of its highest, most developed branch. The height of the typical plant will be given in centimeters.

c) Shoot weight

The shoot weight (fresh and dry weight) will be measured every 30, 60, 90 days until harvest.

c) Root length

Root length will be measured from ground level as well as at 30, 60, 90, and so on until harvesting after sowing. Ten normal seedlings will be chosen from each replication for root measurement. The average root length will be calculated in centimeters.

d) Root weight

The root weight (fresh and dry weight) will be measured every 30, 60, 90 days until harvest.

II. Yield Parameters

a) Number of pods per plant

The total number of pods per plant will be counted at maturity. The number of pods will be expressed as an average of ten plants.

b) Number of seeds per plant

The number of grains in each replication will be recorded and expressed as the average number of grains per pod.

c) Weight of pods per plant

At the time of harvesting, the number of seeds will be determined by the weight of the pods.

d) Weight of 100 seeds per plot

The weight of 100 seeds will be determined after the crop has been harvested. The quality of the seed will be determined by the seed obtained at the end.

e) Total yield

At the time of crop harvesting, the final yield on all plants will be calculated. The following will be used to calculate disease reduction over control:

$$\text{Percent yield increase (\%)} = \frac{\text{Yield of treatment} - \text{Yield of control}}{\text{Yield of control}} \times 100$$

III. Crop studies (Soybean) disease assessment parameters:

The soybean plants that become infected with fungi after germination and at various stages of growth will be collected in order to isolate and identify the fungi that are the cause of the diseases.

To study the effect of all treatments on soil microflora, soil fungi associated with the rhizosphere of soybean plants will be isolated at 30, 60, 90 days, and so on up to harvesting. For this purpose, the soil will be serially diluted, and the appropriate dilution will be inoculated on culture medium. Fungi will be counted and identified to determine their diversity, frequency, and abundance.

5. Statistical analysis

Data will be collected on germination percentage, shoot length, root length, number of pods per plant, number of grains per pod, disease severity, and disease reduction over control. The data will be collected at regular intervals throughout the cropping season, beginning with the appearance of disease and ending with the conclusion of the experiment. ANOVA will be used to analyze the data.

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7. Facilities required.

All the facilities required for the conduct of the experiment are available in School of Agriculture, Abhilashi University, Mandi (H.P.).

8. Collaboration with other Departments/Institutes/Universities

Since all the facilities are available within the university, so no collaboration is needed.

CERTIFICATE

Certified that this synopsis of Ms. Manjula, Registration No. AU21AGPhD0077, entitled **"Biocontrol potential of *Trichoderma viride* on Soybean (*Glycine max* L.) in mid hill condition of Himachal Pradesh"** has been formulated and finalized by the student herself in consultation with the members of Advisory Committee and is in accordance with the instructions of Dean, Faculty of Agriculture, Abhilashi University Mandi (H.P.), on the subject.

Manjula
Signature of the student
(with date)

Signature of the Advisor
(with date)

Advisory Committee:

S.No.	Name and Designation		Signature
1.	Dr. A. K. Gautam Supervisor (Plant Pathology)	Major advisor	
2.	Dr. Ritika Singh Assistant Professor (Genetics and Plant Breeding)	Member	
3.	Dr. Ravinder Assistant Professor (Soil Science)	Member	
4.	Shivani Kaundal Assistant Professor (Biochemistry & Crop Physiology)	Dean's Nominee	

.....
(PG Coordinator,
School of Agriculture)

APPROVED

.....
(Dean, Faculty of Agriculture)

Dean
Faculty of Agriculture
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